

10/501671

DT04 Rec'd PCT/PTO 16 JUL 2004

Sequence Listing

<110> Asahi Kasei Kabushiki Kaisha

<120> High-concentration preparation of soluble thrombomodulin

<130> ASAHI-33

<150> JP2002-009951

<151> 2002-01-18

<160> 9

<210> 1

<211> 516

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble
thrombomodulin

<400> 1

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1

5

10

15

Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu

20

25

30

His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala

35

40

45

Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser

| | | |
|---|-----|-----|
| 50 | 55 | 60 |
| Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly | | |
| 65 | 70 | 75 |
| Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys | | |
| 85 | 90 | 95 |
| Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr | | |
| 100 | 105 | 110 |
| Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn | | |
| 115 | 120 | 125 |
| Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu | | |
| 130 | 135 | 140 |
| Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val | | |
| 145 | 150 | 155 |
| Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg | | |
| 165 | 170 | 175 |
| Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr | | |
| 180 | 185 | 190 |
| Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro | | |
| 195 | 200 | 205 |
| Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys | | |
| 210 | 215 | 220 |
| Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro | | |
| 225 | 230 | 235 |
| Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys | | |
| 245 | 250 | 255 |
| Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala | | |
| 260 | 265 | 270 |
| Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys | | |
| 275 | 280 | 285 |

Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
 290 295 300
 Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
 305 310 315 320
 His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
 325 330 335
 Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
 340 345 350
 Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
 355 360 365
 Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
 370 375 380
 Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
 385 390 395 400
 Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
 405 410 415
 Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
 420 425 430
 Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
 435 440 445
 Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
 450 455 460
 Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile Gly Thr Asp Cys
 465 470 475 480
 Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro
 485 490 495
 Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu
 500 505 510
 Val His Ser Gly

515

<210> 2

<211> 1548

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble
thrombomodulin gene

<400> 2

```
atgcttgggg tcctggtcct tggcgcgctg gccctggccg gcctgggggt ccccgacccc 60
gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctacccg 120
ggccccgcga ctttctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaata 180
acagtgcgct cctcgggtggc tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240
gttggccgcc ggcgccctctg gatcggcctg cagctgccac ccggctgcgg cgaccccaag 300
cgcctcgggc ccctgcgcgg cttccagtgg gttacgggag acaacaacac cagctatagc 360
aggtgggcac ggctcgacct caatggggct cccctctgcg gcccgttgtg cgtcgtgtgc 420
tccgctgttg aggccactgt gccagcgag ccgatctggg aggagcagca gtgcgaagtg 480
aaggccgatg gcttctctg cgagttccac ttcccagcca cctgcaggcc actggctgtg 540
gagccccggc ccgcggctgc cgcgtctcg atcacctacg gcaccccggt cgcgccccgc 600
ggagcggact tccaggcgt gccgggtggc agctccgccg cgggtggctcc cctcggctta 660
cagctaatagt gcaccgcgcc gcccgagcgc gtccaggggc actgggccag ggaggcgccg 720
ggcgcttggg actgcagcgt ggagaacggc ggctgcgagc acgcgtgcaa tgcgatccct 780
ggggtcccc gctgccagtg ccagccggc gccgccctgc aggagacgg gcgctcctgc 840
accgatccg cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900
gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggctggc ggccgaccaa 960
caccggtgcg aggacgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgctgt 1020
```

gtcaacacac aggggtggctt cgagtgccac tgctacccta actacgacct ggtggacggc 1080
 gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140
 ctgaacaaaa ctagctacct ctgcgtctgc gccgagggct tcgcgcccac tccccacgag 1200
 ccgcacaggt gccagatggt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260
 acccaggcta gctgtgagtg ccctgaaggc tacatcctgg acgacgggtt catctgcacg 1320
 gacatcgacg agtgcgaaaa cggcggcttc tgctccgggg tgtgccacaa cctccccggg 1380
 accttcgagt gcatctgcgg gcccgactcg gcccttgtcc gccacattgg caccgac. t 1440
 gactccggca aggtggacgg tggcgacagc ggctctggcg agcccccgcc cagcccgcag 1500
 cccggctcca ccttgactcc tccggccgtg gggctcgtgc attcgggc 1548

<210> 3

<211> 132

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble
thrombomodulin

<400> 8

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Leu | Gly | Val | Leu | Val | Leu | Gly | Ala | Leu | Ala | Leu | Ala | Gly | Leu | Gly |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Phe | Pro | Asp | Pro | Cys | Phe | Arg | Ala | Asn | Cys | Glu | Tyr | Gln | Cys | Gln | Pro |
| | | | | 20 | | | | | 25 | | | | | 30 | |
| Leu | Asn | Gln | Thr | Ser | Tyr | Leu | Cys | Val | Cys | Ala | Glu | Gly | Phe | Ala | Pro |
| | | | | 35 | | | | | 40 | | | | | 45 | |
| Ile | Pro | His | Glu | Pro | His | Arg | Cys | Gln | Met | Phe | Cys | Asn | Gln | Thr | Ala |
| | | | | 50 | | | | | 55 | | | | | 60 | |
| Cys | Pro | Ala | Asp | Cys | Asp | Pro | Asn | Thr | Gln | Ala | Ser | Cys | Glu | Cys | Pro |

65 70 75 80
 Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu
 85 90 95
 Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly
 100 105 110
 Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile
 115 120 125
 Gly Thr Asp Cys
 130

<210> 4

<211> 396

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble
thrombomodulin gene

<400> 4

atgcttgggg tcttggtcct tggcgcgctg gccctggccg gcctgggggtt ccccgacccg 60
 tgcttcagag ccaactgcga gtaccagtgc cagcccctga accaaactag ctacctctgc 120
 gtctgcgccg agggcttcgc gccattccc cagagccgc acaggtgcca gatgttttgc 180
 aaccagactg cctgtccagc cgactgcgac cccaacaccc aggctagctg tgagtgccct 240
 gaaggctaca tcttgacga cggtttcatc tgcacggaca tcgacgagtg cgaaaacggc 300
 ggcttctgct ccggggtgtg ccacaacctc cccggtacct tcgagtgcac ctgcggggccc 360
 gactcgcccc ttgtccgcca cattggcacc gactgt 396

<210> 5

<211> 516

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble
thrombomodulin

<400> 5

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1 5 10 15

Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu

20 25 30

His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala

35 40 45

Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser

50 55 60

Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly

65 70 75 80

Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys

85 90 95

Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr

100 105 110

Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn

115 120 125

Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu

130 135 140

Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val

145 150 155 160

Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg
 165 170 175
 Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr
 180 185 190
 Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
 195 200 205
 Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
 210 215 220
 Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
 225 230 235 240
 Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
 245 250 255
 Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
 260 265 270
 Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
 275 280 285
 Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
 290 295 300
 Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
 305 310 315 320
 His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
 325 330 335
 Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
 340 345 350
 Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
 355 360 365
 Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
 370 375 380
 Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu

385 390 395 400
 Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
 405 410 415
 Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
 420 425 430
 Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
 435 440 445
 Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
 450 455 460
 Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile Gly Thr Asp Cys
 465 470 475 480
 Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro
 485 490 495
 Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu
 500 505 510
 Val His Ser Gly
 515

<210> 6

<211> 1548

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble
 thrombomodulin gene

<400> 6

atgcttgggg tcttggtcct tggcgcgctg gccctggccg gcctgggggt ccccgacccc 60

gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctacccg 120
 ggccccgcga ccttcctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaattg 180
 acagtgcgct cctcggtggc tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240
 gttggccgcc ggcgctcttg gatcggcctg cagctgccac ccggctgcgg cgaccccaag 300
 cgcctcgggc ccctgcgögg cttccagtgg gttacgggag acaacaacac cagctatagc 360
 aggtgggcac ggctcgacct caatggggct cccctctgcg gcccgttgtg cgtcgtgtgc 420
 tccgctgctg agggcactgt gccagcgag ccgatctggg aggagcagca gtgcgaagtg 480
 aaggccgatg gcttcctctg cgagttccac tcccagcca cctgcaggcc actggctgtg 540
 gagccccggc ccgcggctgc cgcgctctcg atcacctacg gcaccccggt cgcgccccgc 600
 ggagcggact tccaggcgct gccgggtggc agctccgccg cgggtggctcc cctcggetta 660
 cagctaattg gcaccgcgcc gcccgagcg gtccaggggc actgggccag ggaggcgccg 720
 ggcgcttggg actgcagcgt ggagaacggc ggctgcgagc acgcgtgcaa tgcgatccct 780
 ggggctcccc gctgccagtg cccagccggc gccgccctgc aggagacgg gcgctcctgc 840
 accgcatccg cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900
 gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggctggc ggccgaccaa 960
 caccggtgcg aggacgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgctgt 1020
 gtcaacacac aggggtggctt cgagtgccac tgctacccta actacgacct ggtggacggc 1080
 gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140
 ctgaacaaa ctagctacct ctgctctgc gccgagggt tcgcgcccatt tccccacgag 1200
 ccgcacaggt gccagatggt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260
 acccaggcta gctgtgagtg ccctgaaggc tacatcctgg acgacggttt catctgcacg 1320
 gacatcgacg agtgcgaaaa cggcggttc tgctccgggg tgtgccacaa cctccccggt 1380
 accttcgagt gcatctgcgg gcccgactcg gcccttgccc gccacattgg caccgactgt 1440
 gactccggca aggtggacgg tggcgacagc ggctctggcg agccccgc cagcccgacg 1500
 cccggctcca ccttgactcc tccggccgtg gggctcgtgc attcgggc 1548

<210> 7

<211> 132

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble
thrombomodulin

<400> 7

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1 5 10 15
Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro
20 25 30
Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro
35 40 45
Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala
50 55 60
Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro
65 70 75 80
Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu
85 90 95
Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly
100 105 110
Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile
115 120 125
Gly Thr Asp Cys
130

<210> 8

<211> 396

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble
thrombomodulin gene



<400> 8

atgcttgggg tcttggtcct tggcgcgctg gccctggccg gcctgggggtt ccccgacccg 60
tgcttcagag ccaactgcga gtaccagtgc cagcccctga accaaactag ctacctctgc 120
gtctgcgccg agggcttcgc gcccatctcc cagcagccgc acaggtgcca gatgttttgc 180
aaccagactg cctgtccagc cgactgcgac cccaacaccc aggctagctg tgagtgcctt 240
gaaggctaca tcttggaaga cggtttcatc tgcacggaca tcgacgagtg cgaaaacggc 300
ggcttctgct ccgggggtgtg ccacaacctc cccggtacct tcgagtgcac ctgcggggccc 360
gactcggccc ttgcccgcca cattggcacc gactgt 396

<210> 9

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic DNA for mutation

<400> 9

aatgtggcgg gcaagggccg a

21